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Quantitative Structure–Activity Studies of Insect Growth Regulators XIV. Three-Dimensional Quantitative Structure–Activity Relationship of Ecdysone Agonists Including Dibenzoylhydrazine Analogs†

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Abstract: *N-tert*-Butyl-*N*,*N*′-dibenzoylhydrazines such as tebufenozide (RH-5992) mimic the action of a molting hormone, 20-hydroxyecdysone, and cause premature molting of larvae leading to their death. Previously, it was shown that one of the benzoyl moieties in dibenzoylhydrazines plays a role similar to that of the aliphatic side chain at the C-17 position of ecdysones. In the present study, *N*-benzoyl-*N*′-benzylhydrazine, *N*,*N*′-dibenzylhydrazine, and *N*-alkanoyl-*N*′-benzoylhydrazine analogs have been synthesized to compare the effects of two carbonyl groups as well as two benzene rings of dibenzoylhydrazine. The quantitative structure–activity relationship of ecdysone agonists including dibenzoylhydrazine analogs was analyzed three-dimensionally using the CoMFA (comparative molecular field analysis) procedure. The CoMFA results suggested that the two carbonyl oxygen atoms of the diacylhydrazine skeleton probably correspond to the oxygens of the 20- and 22-OH groups of ecdysones, and that the benzoyl moiety located closer to the *tert*-butyl group is important for retaining high activity. © 1998 SCI

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Key words: dibenzoylhydrazine; ecdysone; CoMFA; 3-D QSAR; insect growth regulants; cultured integument; *Chilo suppressalis*

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1 INTRODUCTION

Certain dibenzoylhydrazines (**I**: Fig. 1) are active as ecdysone agonists showing the molting hormonal effects on *Drosophila* Kc cells,[1] *Chironomus tentans* cells,[2] and imaginal discs from the Colorado potato beetle (*Leptinotarsa decemlineata* Say) and the greater wax moth (*Galleria mellonella* L.).[3] One of the agonists, tebufenozide [**I**: X_n = 3,5-$(CH_3)_2$, Y_n = 4-C_2H_5] has been commercialized as a selective insecticide against lepidopteran pest larvae.[4] Recently, RH-2485 [**I**: X_n = 3,5-$(CH_3)_2$, Y_n = 2-CH_3-3-OCH_3] has been reported to be another new selective and potent insecticide.[5] External application of these dibenzoylhydrazines causes the premature molting of larvae leading to their death.[6,7]

Previously, we quantitatively analyzed the three-dimensional quantitative structure–activity relationship (3-D QSAR) for a set of variously substituted dibenzoylhydrazines and ecdysone analogs, using the comparative molecular field analysis (CoMFA) procedure[8] to gain insight into the structural requirements for their molting hormonal activity.[9] We suggested that one of the two benzoyl moieties corresponds to the aliphatic side chain at the C-17 position of ecdysones in terms of the molecular mechanism of action. In a more recent study, we demonstrated that replacing the B-ring moiety of dibenzoylhydrazines (**I**, Fig. 1) with an alkyl chain of a certain length retained the molting hormone activity by the synthesis and bioassay of some *N*-alkanoyl-*N'*-*tert*-butyl-*N'*-benzoylhydrazines.[10] In the previous CoMFA study,[9] we made a superposition in which the two oxygens of the 14- and 20-OH groups in ecdysones were matched with two carbonyl oxygens of dibenzoylhydrazines in order to calculate molecular field parameters in a defined lattice space. However, because the 22*S* isomer of the highly active 20-hydroxyecdysone (20E, **II** in Fig. 1) was completely inactive in the receptor-binding assay,[11] we hypothesized that the 22*R*-OH group in the steroidal compounds plays a significant role corresponding to one of the carbonyl oxygens of diacylhydrazines.

In the present work, we synthesized and bioassayed an additional set of analogs such as *N*,*N'*-dibenzyl-, *N*-benzoyl-*N'*-benzyl-, and *N*-alkanoyl-*N'*-benzoylhydrazines to examine the effects of the two carbonyl oxygens and the two benzene rings in dibenzoylhydrazines in more detail. For a set of 62 compounds, including 19 recently and newly synthesized compounds and ecdysone and analogs, the 3-D QSAR was analyzed with CoMFA in which the newly assumed procedure for the molecular superposition described above was examined.

2 MATERIALS AND METHODS

2.1 Chemicals

The compounds used in this study are shown in Table 1 with their melting points. The structures of hydrazine analogs are depicted in Fig. 2, and those of ecdysone analogs in Table 2. Benzyl-benzoyl and dibenzyl compounds (compounds **41**–**43**) and alkanoyl-benzoyl derivatives (**44**, **50**, **51**, **56**–**58**) were newly synthesized in this study according to conventional methods. The synthetic methods for some benzoyl-benzyl and dibenzyl compounds are given below. The structures of newly synthesized compounds were confirmed by [^{1}H]NMR and elemental analysis. [^{1}H]NMR spectra were recorded on a Brucker AC-300 NMR spectrometer at 300 MHz in deuterochloroform ($CDCl_3$) with tetramethylsilane as the internal standard. The NMR spectral data are not shown except for compounds **41**–**43**.

2.1.1 N-tert - *Butyl* -N- *3, 5-dimethylbenzyl*-N'- *4- ethyl-benzoylhydrazine* (***41***)
N-tert-Butyl-*N*-3,5-dimethylbenzoyl-*N'*- 4-ethylbenzoylhydrazine **39** (0·50 g, 1·42 mmol) in anhydrous tetrahydrofuran (THF; 3 ml) was added dropwise to borane-dimethyl sulfide complex (1·0 ml, 10 mmol) dissolved in anhydrous THF (2 ml) with stirring on an ice bath. After stirring overnight at room temperature, methanol was added to the reaction mixture. The solvent was then removed under reduced pressure. The residue was dissolved in ether and washed with 1 M hydrochloric acid, 1 M sodium hydroxide, and brine. After drying the organic layer over anhydrous magnesium sulfate, the solvent was evaporated. The residue was triturated with a mixture of hexane and ethyl acetate to afford *N-tert*-butyl-*N*-3,5-dimethylbenzyl-*N'*-4-ethylbenzoylhydrazine (**41**) (0·12 g, 0·35 mmol) as a colorless powder (yield 25·0%), m.p. 168–169°C. [^{1}H]NMR δ (ppm): 1·20 (3H, t), 1·27 (9H, s), 2·25 (6H, s), 2·64 (2H, q), 3·93 (2H, s), 6·39 (1H, s), 6·83–7·04 (3H, d), 7·13–7·37 (4H, q).

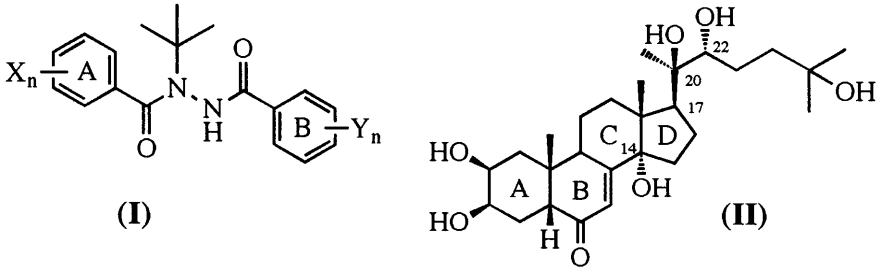
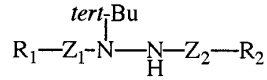


Fig. 1. Structures of ecdysone agonists.

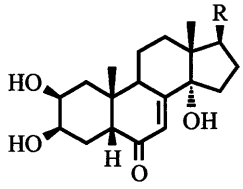
TABLE 1
Molting Hormonal Activity and Physicochemical Properties of Ecdysone Agonists

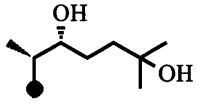
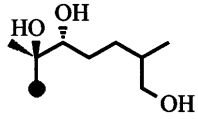
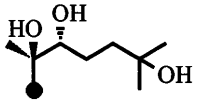
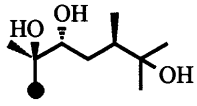
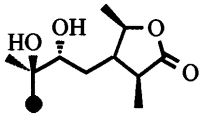
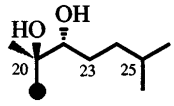


	Compounds				pEC_{50} (M)			
No.	R_1	Z_1	Z_2	R_2	*Obsd*	*Calcd*[a]	*log* P	*m.p.* (°C)
1	C_6H_5	CO	CO	C_6H_5	6·40	6·44	2·45[b]	178–179[c]
2	C_6H_4(2-Cl)	CO	CO	C_6H_5	7·51	6·78	2·59[b]	161–162[c]
3	C_6H_4(2-NO_2)	CO	CO	C_6H_5	6·80	7·29	2·27[b]	181–182[c]
4	C_6H_4(2-CH_3)	CO	CO	C_6H_5	6·49	6·80	2·75[b]	214–215[c]
5	C_6H_4(2-C_6H_5)	CO	CO	C_6H_5	<5·68	6·62	3·60[d]	144–145[c]
6	C_6H_4(2-OCH_3)	CO	CO	C_6H_5	6·50	6·45	2·04[b]	181–182[c]
7	C_6H_4(3-F)	CO	CO	C_6H_5	6·52	6·19	2·78[b]	176–177[c]
8	C_6H_4(3-Cl)	CO	CO	C_6H_5	7·19	6·82	3·28[b]	180–181[c]
9	C_6H_4(3-CF_3)	CO	CO	C_6H_5	6·42	6·46	3·61[b]	203–204[c]
10	C_6H_4(3-NO_2)	CO	CO	C_6H_5	5·64	5·75	2·73[b]	222–223[c]
11	C_6H_4(3-CN)	CO	CO	C_6H_5	5·42	5·85	2·34[b]	173–174[c]
12	C_6H_4(3-CH_3)	CO	CO	C_6H_5	7·04	6·84	2·79[b]	210–212[c]
13	C_6H_4(3-OCH_3)	CO	CO	C_6H_5	6·30	6·60	2·56[b]	188–189[c]
14	C_6H_4(4-Cl)	CO	CO	C_6H_5	6·68	6·47	3·42[b]	212–213[c]
15	C_6H_4(4-I)	CO	CO	C_6H_5	5·85	6·42	3·78[b]	234–235[c]
16	C_6H_4(4-NO_2)	CO	CO	C_6H_5	5·12	4·68	2·63[b]	250–251[c]
17	C_6H_4(4-CN)	CO	CO	C_6H_5	5·12	5·00	2·50[b]	199–200[c]
18	C_6H_4(4-CH_3)	CO	CO	C_6H_5	6·15	6·48	2·99[b]	187–188[c]
19	C_6H_4(4-OCH_3)	CO	CO	C_6H_5	5·21	6·42	2·56[b]	221–222[c]
20	C_6H_4(2-Cl)	CO	CO	C_6H_4(2-F)	7·62	6·72	2·63[b]	191–192[e]
21	C_6H_4(2-Cl)	CO	CO	C_6H_4(2-Cl)	6·62	6·68	2·75[b]	227–228[e]
22	C_6H_4(2-Cl)	CO	CO	C_6H_4(2-Br)	6·61	6·72	2·91[d]	224–225[e]
23	C_6H_4(2-Cl)	CO	CO	C_6H_4(2-CF_3)	5·89	6·19	3·02[d]	218–219[e]
24	C_6H_4(2-Cl)	CO	CO	C_6H_4(2-CH_3)	7·09	6·01	2·91[b]	180–181[e]
25	C_6H_4(2-Cl)	CO	CO	C_6H_4(2-OCH_3)	5·52	5·81	2·37[d]	149–150[e]
26	C_6H_4(2-Cl)	CO	CO	C_6H_4(3-Cl)	7·20	6·99	3·49[b]	144–145[e]
27	C_6H_4(2-Cl)	CO	CO	C_6H_4(3-I)	6·52	6·97	3·87[d]	168–169[e]
28	C_6H_4(2-Cl)	CO	CO	C_6H_4(3-CF_3)	6·00	6·43	3·70[d]	144–145[e]
29	C_6H_4(2-Cl)	CO	CO	C_6H_4(3-NO_2)	5·86	5·39	2·73[d]	180–181[e]
30	C_6H_4(2-Cl)	CO	CO	C_6H_4(3-CH_3)	6·98	7·04	3·11[b]	140–141[e]
31	C_6H_4(2-Cl)	CO	CO	C_6H_4(4-Cl)	7·05	7·11	3·51[b]	179–180[e]
32	C_6H_4(2-Cl)	CO	CO	C_6H_4(4-Br)	7·37	7·05	3·73[d]	219–220[e]
33	C_6H_4(2-Cl)	CO	CO	C_6H_4(4-I)	7·33	7·21	3·96[d]	236–237[e]
34	C_6H_4(2-Cl)	CO	CO	C_6H_4(4-CF_3)	7·08	6·63	3·68[d]	198–199[e]
35	C_6H_4(2-Cl)	CO	CO	C_6H_4(4-NO_2)	6·53	5·93	2·78[d]	216–217[e]
36	C_6H_4(2-Cl)	CO	CO	C_6H_4(4-CN)	5·92	6·49	2·44[d]	190–191[e]
37	C_6H_4(2-Cl)	CO	CO	C_6H_4(4-CH_3)	7·38	7·16	3·15[b]	218–219[e]
38	C_6H_4(2-Cl)	CO	CO	C_6H_4(4-OCH_3)	6·84	6·81	2·82[d]	161–162[e]
39	C_6H_3[3,5-$(CH_3)_2$]	CO	CO	C_6H_4(4-C_2H_5)	8·94	7·97	4·51[d]	194–195[e]
40	C_6H_5	CH_2	CO	C_6H_5	5·10	5·18	3·92[f]	165–166
41	C_6H_3[3,5-$(CH_3)_2$]	CH_2	CO	C_6H_4(4-C_2H_5)	6·06	5·66	5·95[f]	168–169
42	C_6H_3[3,5-$(CH_3)_2$]	CO	CH_2	C_6H_4(4-C_2H_5)	5·76	6·27	5·96[f]	65–66
43	C_6H_3[3,5-$(CH_3)_2$]	CH_2	CH_2	C_6H_4(4-C_2H_5)	4·89	4·72	6·09[f]	Liquid
44	C_6H_3[3,5-$(CH_3)_2$]	CO	CO	*n*-C_3H_7	5·53	6·51	2·60[f]	162–163
45	C_6H_3[3,5-$(CH_3)_2$]	CO	CO	*n*-C_4H_9	7·10	7·25	3·13[f]	91–92[g]
46	C_6H_3[3,5-$(CH_3)_2$]	CO	CO	*n*-C_5H_{11}	8·05	7·61	3·66[f]	144–145[g]
47	C_6H_3[3,5-$(CH_3)_2$]	CO	CO	*i*-C_5H_{11}	7·97	7·40	3·53[f]	136–137[g]
48	C_6H_3[3,5-$(CH_3)_2$]	CO	CO	*n*-C_6H_{13}	8·13	7·71	4·19[f]	115–116[g]
49	C_6H_3[3,5-$(CH_3)_2$]	CO	CO	*i*-C_6H_{13}	7·96	7·66	4·06[f]	161–162[g]
50	C_6H_3[3,5-$(CH_3)_2$]	CO	CO	*n*-C_7H_{15}	5·16	7·15	4·72[f]	89–90
51	*n*-C_4H_9	CO	CO	C_6H_4(4-C_2H_5)	5·40	5·36	3·16[f]	145–146
52	*n*-C_5H_{11}	CO	CO	C_6H_4(4-C_2H_5)	5·09	5·14	3·69[f]	112–113[g]
53	*i*-C_5H_{11}	CO	CO	C_6H_4(4-C_2H_5)	5·34	6·08	3·56[f]	129–130[g]
54	*n*-C_6H_{13}	CO	CO	C_6H_4(4-C_2H_5)	5·17	5·23	4·22[f]	140–141[g]
55	*i*-C_6H_{13}	CO	CO	C_6H_4(4-C_2H_5)	5·04	5·14	4·09[f]	156–157[g]
56	*cyc*-C_4H_7	CO	CO	C_6H_4(4-C_2H_5)	4·14	4·32	2·49[f]	190–191
57	*cyc*-C_6H_{11}	CO	CO	C_6H_5	4·71	4·39	2·58[f]	212–213
58	*cyc*-C_6H_{11}	CO	CO	C_6H_4(4-C_2H_5)	5·06	4·71	3·60[f]	210–211
59	Ecdysone[h]				<5·27	6·72	−0·36[f]	[i]
60	20-Hydroxyecdysone[h]				6·75	6·70	−1·72[f]	[i]
61	Cyasterone[h]				6·37	6·02	−1·97[f]	[j]
62	Inokosterone[h]				6·18	6·48	−1·50[f]	[j]
63	Makisterone[h]				5·73	6·32	−1·32[f]	[j]
64	Ponasterone[h]				7·53	6·88	0·49[f]	[j]

[a] Calculated by eqn (1).
[b] Experimentally measured (from Refs 23 and 24).
[c] From Ref. 23.
[d] Estimated empirically (from Refs 23 and 24).
[e] From Ref. 24.
[f] Calculated by CLOGP method.
[g] From Ref. 10.
[h] Structures are shown in Table 2.
[i] Purchased from Sigma Chemical Company.
[j] Gift from Nippon Kayaku Co., Ltd.

TABLE 2
Structures of Ecdysone Analogs



No.	*Name*	*R*	*No.*	*Name*	*R*
59	Ecdysone		**62**	Inokosterone	
60	20-Hydroxyecdysone		**63**	Makisterone A	
61	Cyasterone		**64**	Ponasterone A	

2.1.2 N-tert-*Butyl*-N′-*9-fluorenylmethoxycarbonyl-hydrazine*

N - (9 - Fluorenylmethoxycarbonyl)succinimide (5·42 g, 16·1 mmol) suspended in dioxane was added dropwise to a mixture of *tert*-butylhydrazine hydrochloride (2·00 g, 16·1 mmol) and sodium hydrogen carbonate (1·4 g, 16·7 mmol) in 1,4-dioxane + water (2 + 1, by volume; 20 ml) with stirring on an ice bath. After stirring for 3 h at room temperature, dioxane was removed under reduced pressure. The residue was extracted with ether, washed once with brine, and then dried over anhydrous magnesium sulfate. The solvent was evaporated to give a solid residue. The residue was treated with a mixture of hexane and ethyl acetate to afford *N-tert*-butyl-*N′*-9-fluorenylmethoxycarbonylhydrazine (4·7 g, 15·1 mmol) as a colorless powder (yield 94%).

2.1.3 N-tert - *Butyl* -N - *3, 5 - dimethylbenzoyl* - N′ - *9-fluorenylmethoxycarbonylhydrazine*

3,5-Dimethylbenzoyl chloride (1·5 g, 8·90 mmol) and triethylamine (3·50 g, 34·6 mmol) were simultaneously added dropwise to a suspension of *N-tert*-butyl-*N′*-9-fluorenylmethoxycarbonyldrazine (2·35 g, 7·57 mmol) in anhydrous diethyl ether (50 ml) with stirring on an ice bath. After stirring overnight at room temperature, the reaction mixture was diluted with ether (100 ml) and washed successively with 1 M hydrochloric acid and brine. The organic layer was dried over anhydrous magnesium sulfate, and the solvent was evaporated. The residue was treated with a mixture of hexane and ethyl acetate to afford *N-tert-N*-3,5-dimethylbenzoyl-*N′*-9-fluorenylmethoxycarbonylhydrazine (2·81 g, 6·35 mmol) as a colorless powder (yield 83·9%).

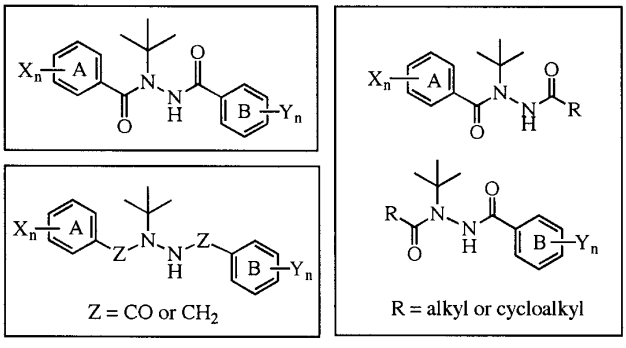


Fig. 2. Hydrazine derivatives used for CoMFA.

2.1.4 N-tert-*Butyl*-N-*3,5-dimethylbenzoylhydrazine*

N-tert - Butyl-*N*-3, 5 - dimethylbenzoyl -*N′*- 9 - fluorenylmethoxycarbonylhydrazine (2·43 g, 5·49 mmol) in dimethylformamide (DMF; 10 ml) was added dropwise to piperidine (0·46 g, 5·40 mmol) dissolved in DMF (3 ml) with stirring at room temperature. After stirring for 1 h at room temperature, DMF was removed under reduced pressure. The residue was purified by silica-gel column chromatography with hexane + ethyl acetate (3 + 1 by volume) to afford *N-tert*-butyl-*N*-3,5-dimethylbenzoylhydrazine (1·20 g, 5·45 mmol) as a colorless powder (yield 99·2%).

2.1.5 N-tert-*Butyl*-N-(*3,5-dimethylbenzoyl*)-N′-(*4-ethylbenzyliden)hydrazine*

4-Ethylbenzaldehyde (0·72 g, 5·37 mmol) dissolved in 2 ml of anhydrous THF was added dropwise to *N-tert*-butyl-*N*-3,5-dimethylbenzoylhydrazine (1·20 g, 5·45 mmol) with stirring at room temperature. After stirring overnight at 50°C, THF was removed under reduced pressure and the residue was extracted with

ether. The ether phase was washed once with brine, then dried over anhydrous magnesium sulfate. The solvent was evaporated to give a solid residue. The residue was manipulated with a mixture of hexane and ethyl acetate to afford light yellowish crystals (1·58 g, 4·70 mmol) of *N-tert*-butyl-*N*-(3,5-dimethylbenzoyl)-*N′*-(4-ethylbenzyliden)hydrazine (yield 86·2%).

2.1.6 N-tert-*Butyl*-N-(*3,5-dimethylbenzoyl*)-N′-(*4-ethylbenzyl*)*hydrazine* (***42***)
After dissolving *N-tert*-butyl-*N*-(3,5-dimethylbenzoyl)-*N′*-(4-ethylbenzyliden)hydrazine synthesized above (1·34 g, 3·98 mmol) in methanol with a small amount of bromocresol green, sodium cyanoborohydride (0·40 g, 6·37 mmol) in methanol was added dropwise with stirring at room temperature. During the addition, the solution was maintained at pH 3 to 4 with 1 M hydrochloric acid. After the addition was over, stirring was continued for 1 h at room temperature. Methanol was removed under reduced pressure, and the residue was dissolved in ether and washed once with brine. After drying the solution over anhydrous magnesium sulfate, the solvent was evaporated to give a solid residue. The residue was manipulated with a mixture of hexane and ethyl acetate to afford *N-tert*-butyl-*N*-3,5-dimethylbenzoyl-*N′*-4-ethylbenzylhydrazine (1·20 g, 3·55 mmol) as a colorless powder (yield 89·2%). [^{1}H]NMR δ (ppm): 1·83 (3H, t), 1·52 (9H, s), 2·33 (6H, s), 2·58 (2H, q), 3·74 (2H, br) 4·13 (1H, br), 6·74 (7H, m).

2.1.7 N-tert-*Butyl*-N-(*3, 5-dimethylbenzyl*)-N′-(*4-ethylbenzyl*)*hydrazine* (***43***)
N-tert-Butyl-*N*-3,5-dimethylbenzoyl-*N′*- 4- ethylbenzylhydrazine (1·05 g, 3·10 mmol) in anhydrous THF was added dropwise to borane-dimethyl sulfide complex (1·0 ml, 10 mmol) in anhydrous THF (5 ml) with stirring on an ice bath. After stirring for 3 h at room temperature, methanol was added and the solvent was then removed under reduced pressure to afford an oil. After dissolving the oil residue in ether and washing successively with 1 M hydrochloric acid, 1 M sodium hydroxide, and brine, the organic layer was dried over anhydrous magnesium sulfate. The solvent was evaporated to give a yellowish oil. The residue was purified by silica-gel column chromatography with hexane + ethyl acetate (40 + 1 by volume) to afford *N-tert*-butyl-*N*-3,5-dimethylbenzyl-*N′*-4-ethylbenzylhydrazine (0·56 g, 1·73 mmol) as a yellowish oil (yield 55·7%). [^{1}H]NMR δ (ppm): 1·18 (9H, s), 1·19 (3H, t), 2·34 (6H, s), 2·59 (2H, q), 3·32 (2H, s), 3·68 (2H, s), 6·89–7·06 (7H, m).

2.2 Bioassay

The molting hormonal activity of the test compounds, as the activity to promote the chitin synthesis for the new cuticle, was evaluated using the cultured integument system, as reported earlier.[10,12–14] Briefly, integument fragments excised from the diapause larvae of rice stem borers (*Chilo suppressalis* Walker) were floated on a medium containing each of the test compounds at various concentrations and cultured at 28(±3)°C for 24 h. In each experimental run, eight integument fragments from different larvae were considered as a group. The control group was treated with 1 ml litre^{-1} dimethyl sulfoxide, and another group with 20E (1·0 mg litre^{-1}) which served as the positive standard. The treated integument fragments were transferred to fresh medium (1 ml per well) containing [^{14}C]GluNAc (*c.* 18 000–24 000 dpm ml^{-1} medium) and further cultured for three days. The integument fragments were then washed three times with distilled water, and the radioactivity incorporated into the fragments was measured in Aquasol II (NEN Du Pont, Boston, MA, USA) by a liquid scintillation counter. From the concentration–response relationship, the 50% effective concentration EC_{50} (M), was estimated for each compound using probit analysis.[15,16] The logarithm of the reciprocal EC_{50}, the pEC_{50} listed in Table 1, was used as an index of the molting hormonal activity. Except for newly assayed compounds, the pEC_{50} values were taken from our previous publications.[9,10,14]

2.3 CoMFA procedure

2.3.1 Molecular modeling
All computations were performed with the molecular modeling software package SYBYL ver. 6.3 (Tripos Co., St. Louis, MO). The conformation of dibenzoylhydrazines was deduced from the X-ray diffraction data of *N-tert*-butyl-*N*,*N′*-dibenzoylhydrazine (**1**) and the 2-chlorobenzoyl analog (**2**),[9] and fully optimized by the semi-empirical molecular orbital method, PM3,[17,18] in the program package MOPAC 5.0.[19,20] The structure of 20E was also constructed based on its reported X-ray structure[21] and was fully optimized by PM3 as reported previously.[9] The skeletal conformation of other diacylhydrazines and benzylhydrazines was constructed from the comparable dibenzoylhydrazines and fully optimized by PM3. The fully optimized conformation was regarded as the 'active' conformer of each compound. The coordinates of the optimized structure were calculated by use of the SYBYL standard values for bond lengths and angles. The charge for each atom in the compounds was calculated using PM3[17,18] and AM1.[22]

2.3.2 CoMFA procedure
The analyses were carried out using the QSAR option of the SYBYL. The lattice spacing was 2 Å and the +1 charge and the sp^3 carbon were used as probes to estimate the electrostatic and steric molecular fields, respectively. The molecules were superposed in the lattice

space prepared automatically with the default setting. The electrostatic and steric potential energies at each lattice point were calculated using Coulombic and Lennard–Jones potential functions, respectively. The hydrophobic effect was evaluated using the molecular hydrophobicity, log *P*, as the lattice-independent external descriptor. The log *P* values of compounds were either measured experimentally,[23,24] estimated empirically,[23,24] or calculated using the CLOGP method.[25,26] The correlations of the biological activity index with the lattice variables and log *P* were analyzed by the partial least squares (PLS) method[27,28] setting the column filtering as 2 kcal mol^{-1}. The CoMFA results were represented by the leave-one-out cross-validated correlation coefficient, *q*, and standard deviation, s_{press}, and the number of optimum components, *m*, as well as the conventional correlation coefficient, *r*, and standard deviation, *s*, in addition to the weight percentage of the type of the descriptors participating in the correlation. The results were visualized by diagrams for contour lines connecting lattice points having an equivalent coefficient level for each molecular field surrounding a set of superposed molecules.

2.3.3 Molecular superposition

In all superposition procedures, the sum of the squares of distance between corresponding or matched atoms was minimized. For the superposition of the dibenzoylhydrazines (**1**–**39**) and four alkanoyl-benzoyl analogs having *n*-propyl (**44**), *cyc*-butyl (**56**), and *cyc*-hexyl (**57**, **58**) groups, eight atoms in the skeletal structure **-CC(=O)NNC(=O)C-** were selected. For other alkanoyl-benzoyl analogs (**45**–**55**) having a C4 or longer alkyl chain, the 4th chain carbon from the CO group and the carbon at the *para* position of the benzene ring of dibenzoylhydrazines were matched with each other instead of the corresponding α(first)-carbon atoms. For the benzoyl-benzyl and dibenzyl derivatives (**40**–**43**), the skeletal 'chain' atoms, **-CC(=O)NNC-C-** (seven atoms) and **-CCNNCC-** (six atoms), were used for the superposition onto the corresponding atoms in dibenzoylhydrazines. The procedures are illustrated in Fig. 3. In these procedures, the N atoms bearing the *tert*-butyl group were always taken to correspond with one another.

The superposition of ecdysone analogs was made by selecting every corresponding atom in the steroidal

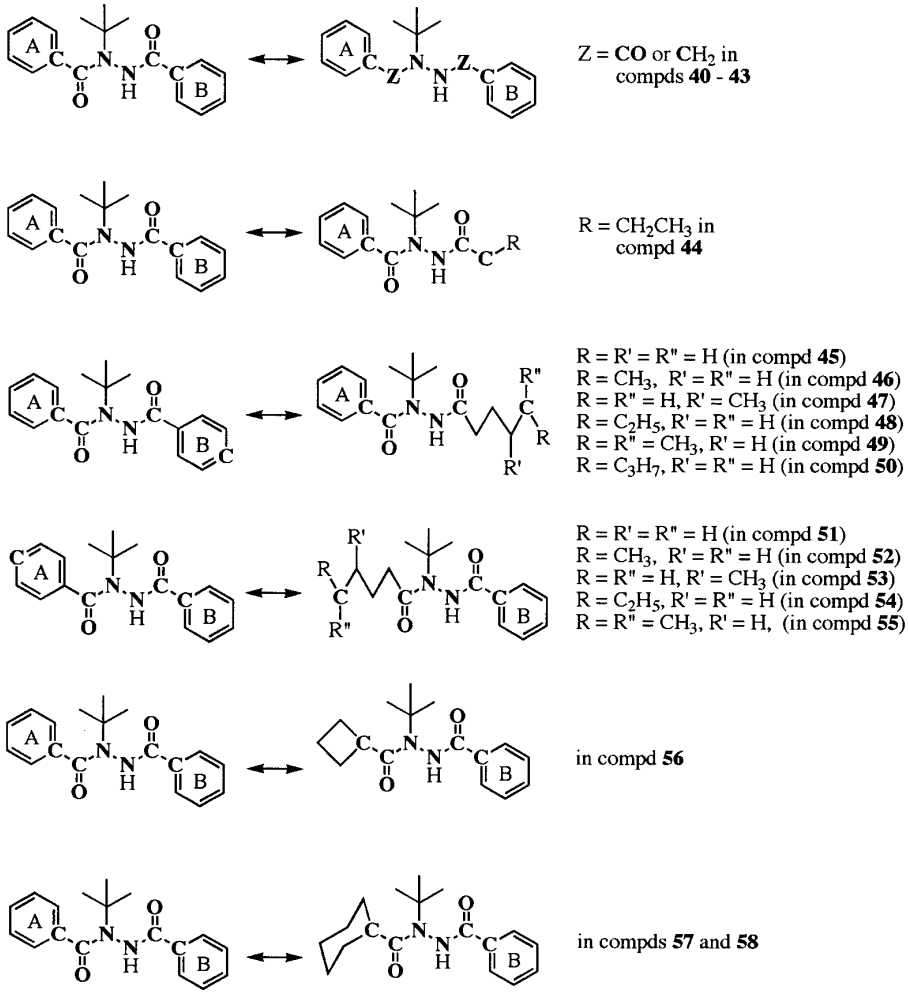


Fig. 3. Superposition procedures between dibenzoylhydrazines and other hydrazine derivatives. In each structural pair, corresponding atoms designated in bold were matched.

skeleton and substituents as well as the C-17 side chain up to the C-24 atom. The structural differences beyond C-24 for compounds **59–64** were not considered for the superposition. The superposition of the set of ecdysones with non-steroidal compounds was examined by four procedures shown in Fig. 4. The two carbonyl oxygen atoms of dibenzoylhydrazines were matched with the 20- and 22-OH oxygens of ecdysones in Superpositions-a and -b, and with the 14- and 20-OH oxygens in Superpositions-c and -d. As indicated in Fig. 4, five atoms denoted with numerals, 1, 3, 4, 5 and 7 in dibenzoylhydrazines fitted to the corresponding atoms in ecdysones in Superpositions-a and -b. Four atoms, 1, 2, 4 and 6 were used in Superpositions-c and -d. These procedures were selected by trial examinations for best superpositions while retaining the mutual correspondence between two carbonyl oxygens of diacylhydrazines and two hydroxy oxygens of ecdysone analogs. The stereo views for Superpositions-a to -d between tebufenozide and 20E are shown in Fig. 5.

3 RESULTS

The activities of newly synthesized alkanoylbenzoylhydrazines having cycloalkyl groups at the A-ring position (**56–58**) were lower than those of analogs having chain alkyl groups (**51–55**) which, in turn, were lower than those of the corresponding 'B-ring' counterparts (**45–49**). At the B-ring position, the elongation of the alkyl chain from C6 (**48**) to C7 (**50**) drastically decreased the activity. The activity of compounds (**1**, **39**) was lowered to 1/20 (**40**) ~1/1000 (**41**, **42**) in compounds in which one of the carbonyl groups was reduced to CH_2. For the dibenzyl compound (**43**), in which both CO groups were reduced, the activity decreased further to the level of 1/10 000 of that of the dibenzoyl compound (**39**). These molting hormonal activity values as well as those for previously reported compounds[9] are listed in Table 1.

The CoMFA results derived from four superposition procedures using 62 compounds are listed in Table 3.

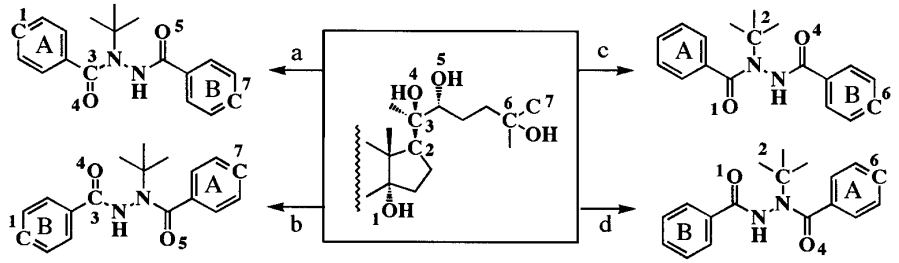


Fig. 4. Superposition procedures between dibenzoylhydrazines and 20E. In each of four procedures, atoms having the corresponding numeral were matched.

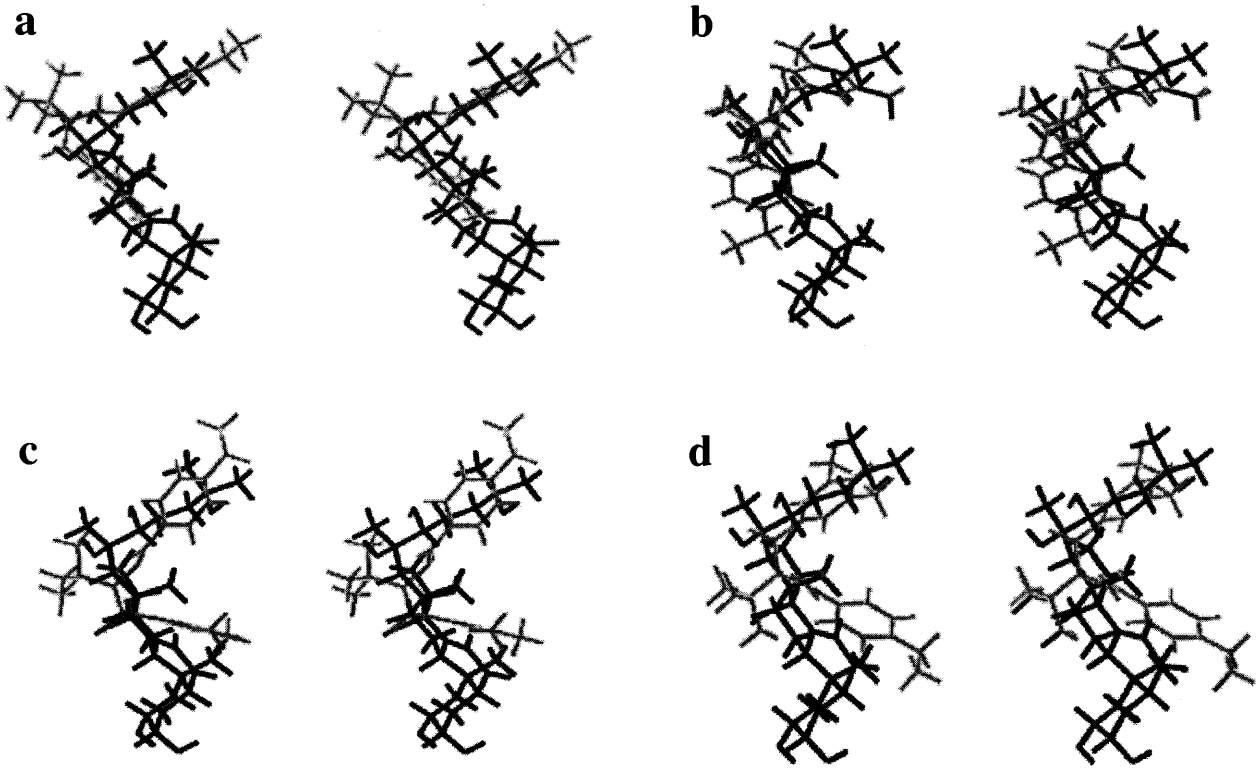


Fig. 5. Stereoviews for Superpositions-a, -b, -c and -d between tebufenozide (**39**) drawn with shaded bonds and 20E (**60**) with solid bonds.

TABLE 3
CoMFA Correlation Statistics[a]

	Cross-validated		*Conventional*			*Contribution* (%)			
Superposition	q^2	s_{press}	r^2	s	m	*st.*[b]	*el.*[c]	*log* P	*eqn no.*
a	0·409	0·794	0·730	0·537	4	47·2	47·3	5·6	(1)
b	0·359	0·827	0·729	0·537	4	47·5	45·4	7·0	(2)
c	0·307	0·852	0·603	0·645	3	53·1	38·1	8·9	(3)
d	0·388	0·801	0·616	0·634	3	49·4	44·0	6·6	(4)

[a] The number of compounds used in the analysis is 62.
[b] From sum of contributions of the steric field descriptors.
[c] From sum of contributions of the electrostatic field descriptors.

Superposition-a was best in terms of both the cross-validated and conventional correlation statistics in eqn (1), despite the quality being not as high as one would like. If the external log *P* term was not introduced in the analysis, the correlation quality was lower ($q^2 = 0{\cdot}388$, $s_{press} = 0{\cdot}807$) in eqn (1). The HINT (hydropathic interaction) procedure developed by Kellogg *et al.*[29] to examine participations from the hydrophobic molecular field was not appropriate in this analysis, because the experimentally observed log *P* values of some dibenzoylhydrazines differed greatly from those estimated in the HINT procedure. Using atomic charges calculated by PM3[17,18] gave better results than those determined by AM1[22] in eqn (1).

Plate 1-I shows contour maps of the steric and electrostatic potentials according to eqn (1) with tebufenozide (**39**) inserted. Sterically permissible regions are observed to cover each of the 2-, 3-, and 5-positions of the A-ring moiety and the 4-position of the B-ring as shown in Plate 1-Ia. A sterically forbidden region is developed outside the permissible region corresponding to the 4-position of the B-ring. In addition, forbidden regions appeared below the A-ring, along the *tert*-butyl group, and surrounding the 4-position of A-ring and the 2,3-positions of the B-ring. In Plate 1-Ib, positive electrostatic-potential regions are shown to surround the 3-, 4- and 5-positions of the A-ring moiety as well as the 2-, 3-, 4- and 5-positions of the B-ring. A large negative electrostatic potential network appears to accommodate the A-ring as well as the adjacent carbonyl group.

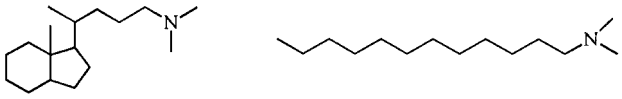
In Plate 1-II, 20E (**60**) was inserted into the steric and electrostatic contour maps according to eqn (1). The sterically forbidden regions found apart from the 4-substituent position of the A-ring and appearing near the *tert*-butyl group of dibenzoylhydrazines in Plate 1-Ia seem to be located in the neighborhood of the carbonyl group in the sterol B-ring and in the direction which the 20-CH_3 group would be extended, respectively, in Plate 1-IIa. The forbidden region below the A-ring of dibenzoylhydrazines corresponds with the region below the D-ring of ecdysones. The sterically permissible regions surrounding the 2-, 3- and 5-positions of the A-ring of dibenzoylhydrazines seem to accommodate the sterol C- and D-rings. In Plate I-IIb, electronegative regions surround the 20-OH group and the space between the 22-OH and 14-OH groups. Electropositive regions are located at the C-17 side chain terminal and at the periphery of the C-ring of ecdysones.

4 DISCUSSION

As mentioned above, our recent work indicated that the B-ring moiety of dibenzoylhydrazines can be replaced by a saturated alkyl chain without losing much of the molting hormonal activity.[10] Thus, the B-ring moiety may participate in the mechanism, triggering the activity with a role similar to that of the C-17 aliphatic side chain of ecdysones. The activities of the A-ring analogs having longer alkyl chains (**51**–**55**) are significantly lower than those of the B-ring counterparts (**45**–**49**). Even when the chain was replaced with *cyc*-butyl (**56**) and *cyc*-hexyl group (**57**, **58**) to make the A-ring moiety compact, the activity was not recovered. Because the size of the *cyc*-hexyl group is almost equivalent to that of the phenyl,[30] the aromatic ring should be important for the activity at the A-ring region.

Because the number of non-steroidal compounds outweighs that of ecdysone analogs, the CoMFA contour maps seem to confirm the structure–activity pattern of non-steroidal analogs better than that of ecdysones. However, the maps appear not to be incompatible with the structure–activity pattern of ecdysone analogs in general. Around the 2(*ortho*)-position of the A-ring of dibenzoylhydrazines, no sterically restricted space is shown in Plate 1-Ia. This is unexpected, because the activity of the 2-Ph analog (**5**) is undetectably low and not included in the analysis. The phenyl substituent may protrude into a forbidden space which does not appear explicitly in Plate 1-IIIa. The reason why the activity of ecdysone (**59**) was unpredictably low may be due to the lack of the OH group at the 20-position.

The sterically forbidden region appearing outside the permissible space corresponding to the 4(*para*)-substituent position of the B-ring of dibenzoylhydrazines is



(III) (IV)

Fig. 6. Structures of molting inhibitors, *N*,*N*,*δ*,7α-tetramethyl-octahydro-1-*H*-indene-1-butanamine (**III**) and *N*,*N*-dimethyl-dodecamine (**IV**).

reasonable because the activity of compounds having longer alkyl chains instead of the B-ring is lower. This, in addition to the forbidden regions outside the permissible regions around the A-ring substituents, seems not inconsistent with our classical QSAR for the larvicidal[23,24] and molting hormonal activities of dibenzoylhydrazine analogs.[14] Substituents with too much bulk are unfavorable to these activities, anyway. At the *tert*-butyl position, every compound except for steroidal ecdysones has the *tert*-butyl group. With skeletally different ecdysone analogs and conformationally modified benzyl compounds included in this analysis, the sterically restricted situation of this region could be disclosed. This result is compatible with earlier observations that the *tert*-butyl group is the best substituent at this position.[6]

The activities of compounds **19**, **24**, **50** deviate markely from those predicted by eqn (1), as shown in Table 1. When these compounds were omitted from the analysis, the correlation was greatly improved ($r^2 = 0{\cdot}840$, $s = 0{\cdot}413$, optimum component = 4), but the sterically unfavorable region at the terminal of the aliphatic side chain in steroids or at the end of *para*-substituent position of the B-ring of dibenzoylhydrazines disappeared. Other fields were similar to those derived from eqn (1). The end of the substituents of these compounds may project into forbidden regions (in compounds **19** and **50**) or permissible regions (in compound **24**) not clearly proposed by the CoMFA model for the present set of compounds.

In our previous CoMFA study,[9] the 14-OH oxygen atom of ecdysones was superposed on one of the CO oxygens of dibenzoylhydrazines, because the 14-deoxy steroids are less active in whole animals than their 14-OH counterparts.[31,32] The binding activity of 20E with the receptor preparation is, however, enhanced remarkably by omitting the 14-OH group,[33] and decreased by converting the configuration of the 22-OH group,[11] mentioned above. Moreover, the molting hormonal activity of ecdysone (**59**) is reduced by deleting the 22-OH group *in vivo*.[31] Thus, it seemed that the 22-OH group is more important than the 14-OH group. The CoMFA results shown in Table 3 indicated that Superposition-a, in which the two carbonyl oxygens of dibenzoylhydrazines correspond to the 20 and 22-OH oxygens in steroidal hormones, is in accord with the above findings.

Hsu *et al.*[34] suggested recently that the X-ray structure is most likely as the receptor recognition conformer from the conformational analysis, while other conformations need to be considered in the binding model. According to their calculation, the greatest solvation stabilization is achieved in the X-ray structure but the least gained in the fully extended conformation among possible conformations.

Structure–activity relationship studies for steroidal ecdysone analogs have been carried out, suggesting that there are three important regions in ecdysones involved in the receptor binding.[35–37] The first region is the steroidal A-ring which fits in a pocket at the receptor site. A particularly strong binding occurs at the second region, defined as encompassing the 6-keto-7-ene structural unit and the 14-OH group. The third region is the side chain with a specific binding for the 22-OH group. The interaction with the other part of the side chain has been less defined. The structural variations in these studies may not be enough to completely explain the ligand–receptor interactions for ecdysone agonists including dibenzoylhydrazines. The present study suggests that the A- and B-rings of ecdysones including the 6-keto-7-ene structural unit seem to be unnecessary for the molting hormonal activity. The present set of substituents on the A-ring of dibenzoylhydrazines included in the CoMFA was unable to propose any electronegative potential zone for the 6-keto group of ecdysones. Instead, the electropositive potential network seems to traverse the B-ring, as shown in Plate 1-IIb. In this respect, it is interesting to note that compounds such as **III** and **IV** shown in Fig. 6 have been observed to show insecticidal activity with inhibition of molting.[38] The CoMFA result has to be considered as being inconclusive, but useful for obtaining clues for designing 'simplified' as well as optimized structures for further synthetic challenges.

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